167. A method of staining target chromosomal DNA to detect in an interphase cell one or more genetic translocations identified with chromosomal abnormalities, said method comprising:

(a) providing a heterogeneous mixture of two or more labeled nucleic acid probes having a combined complexity of about 50 kb, which probes contain nucleic acid segments which are substantially complementary to nucleic acid segments that flank and/or extend partially or fully across breakpoint regions known to be associated with genetic translocations;

(b) reacting the heterogeneous mixture with the targeted chromosomal DNA by in situ hybridization; and

(c) observing the proximity or overlap of the regions stained by each probe, to determine whether said translocation is present in the interphase cell.--

REMARKS

Entry of the foregoing and further and favorable reconsideration of the subject application pursuant to and consistent with 37 C.F.R. §1.112 is respectfully requested.

By the present amendment, new claims 162-167 have been added. These claims are directed to the same subject matter as claims 131, 144, 147, 148, 150 and 155, but recite that the probe complexity is "about 50 kb," rather than "at least

40 kb." These claims derive support from throughout the specification and claims as originally filed. No new matter has been added.

Turning now to the Official Action, claims 131-135 and 137-161 are rejected under 35 U.S.C. §112, first paragraph, as purportedly unsupported by the specification as originally filed. This rejection, to the extent that it applies to the claims as amended, is respectfully traversed.

The Examiner continues to assert that the recitation in the present claims of probes with at least 40 kb of complexity introduces new matter into the claims. In their previous response, Applicants pointed to specific examples of support for the "at least 40 kb" limitation. In response the Examiner argues, at page 2 of the Official Action, that "all of these citations which clearly cite complexity also cite 50 kb of complexity as the closest numeric value to the present 40 kb in the instant claims." Applicants respectfully disagree, and maintain that the present specification provides ample explicit support for probes with a complexity of at least 40 kb. At page 13, lines 6-7 of the specification, Applicants note that "prior to this invention, probes employed for in situ hybridization techniques had complexities below **40 kb**, and more typically on the order of a few kb." At page 37, lines 24-26, Applicants point out that "from **about a 40 kb** to about a 100 kb target sequence may be presently necessary to provide a reliable, easily detectable signal." At page 38, lines 5-13, Applicants reiterate that,

The term "complexity" therefore refers to the complexity of the total probe no matter how many visually distinct loci are to be detected, that is, regardless of the distribution of the target sites over the genome.

As indicated above, with current hybridization techniques it is possible to obtain a reliable, easily detectable signal with a probe of **about 40 kb** to about 100 kb (eg. the probe insert capacity of one or a few cosmids) targeted to a compact point on the genome. Thus, for example, a complexity in the range of approximately 100 kb now permits hybridization to both sides of a tumor-specific translocation.

Further support may be found in the discussion of the use of mixtures of low complexity probes at page 40, lines 12-22:

> One method of forming the probes of the present invention is to pool many different low complexity probes. Such a probe would then comprise a "heterogenous mixture" of individual clones sequences. The number of clones required depends on the extent of the target area and the capacity of the cloning vector. If the target is made up of several discrete, compact loci, that is, single spots at the limit of microscopic resolution, then **about 40 kb**, more preferably 100 kb, for each spot gives a reliable signal given current techniques. The portion of the probe for each spot may be made up from, for example, a single insert from a yeast artificial chromosome (YAC), from several cosmids each containing 35-**40 kb** of probe sequence, or from about 25 plasmids each with 4 kb of sequence.

These citations from the instant application all cite the numerical value of 40 kb of complexity.

In response to Applicants citations from their previous response, the Examiner asserts that

> The one citation that gives 40 kb as a numeric value for a probe on page 38, lines 8-11, lacks any complexity wording. It is well known in the art that a citation such as 40 kb is first interpreted as being a length rather than a complexity.

J.

Without taking a position on the Examiner's conclusion about what is "well known in the art," Applicants respectfully point out that the citation at page 38 falls at the end of a two-page discussion in the specification of probe complexity. Nevertheless, assuming *arguendo* that the Examiner is correct, that a term such as "40 kb" in isolation would be interpreted to refer to length rather than complexity, even a casual reading of the text surrounding the passages cited above shows that the application, in at least those sections, is indisputably referring to probe complexity. The fact that, at other locations in the specification the term "kb" is used to refer to length is irrelevant.

In view of the foregoing, Applicants maintain that the pending claims fully comply with the requirement of 35 U.S.C. §112. Withdrawal of this rejection is thus respectfully requested. Furthermore, Applicants note that claims 145 and 154 are subject to the present rejection, despite the fact that they recited probes no shorter than "about 50 kb," the complexity that the Examiner agrees is supported by the present specification. Applicants respectfully suggest that, even if the Examiner does not find the foregoing arguments persuasive, nonetheless the rejection of claims 145 and 154 should be withdrawn as it cannot reasonably apply to these two claims.

From the foregoing, further and favorable reconsideration in the form of a Notice of Allowance is believed to be next in order and such action is earnestly solicited.

In the event that there are any questions concerning this amendment, or the application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Malcolm K. McGowan, Ph.D.
Registration No. 39,300

Post Office Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: October 1, 1999